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IMMUNIZATION TESTS WITH GLANDERS VACCINE.

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INTRODUCTORY.

Among the diseases of horses with which the veterinary authorities are concerned glanders is probably the most important, and unless strict measures for its control are enforced the tendency of the disease is to spread more or less rapidly. This fact is due to the character of the disease, to the prevailing methods of caring for horses, and, probably more important than all, to the frequent latent existence of the disease in apparently healthy animals. The destruction of all infected animals has been accepted as a matter of course in all civilized countries, and owing to the dangerous character of the disease and the possibility of transmission to man, this action appears to be the sanest and most reasonable procedure in its control. On the other hand, the possibility of a method of immunization of healthy animals is worthy of consideration and would be of great advantage.

Ever since the discovery of mallein as a diagnostic agent for glanders, experiments have been conducted by various investigators relative to its immunizing and curative value. Many favorable reports have been made by veterinarians of the results obtained. On the contrary, others appear to have had no satisfaction from its use. Since it has been proved that cases of glanders may recover it is rather difficult to establish the value of the immunizing agents as to their action on the disease. Fortunately, we now possess a means by which the presence of immune bodies can be demonstrated in the animal upon which attempts at immunization are made. With the serological tests at our command we may control to some extent the action of an immunizing substance and observe how long the immune bodies are present in an animal receiving immunization treatment.

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It is unfortunate, however, that the demonstration of immune bodies does not indicate the degree of immunity in the animals.

We may obtain in glanders immunization an agglutination value of 1 to 5,000 or over or a complement fixation with 0.02 of a cubic centimeter of serum which may continue for a period of several months, yet this same animal, which apparently is supplied with a great amount of immune bodies, can be readily infected with glanders bacilli. Therefore, in tests undertaken for establishing the degree of immunity against glanders in the horse, it is necessary to expose the injected animals to an infection such as occurs under natural conditions. Observations of such animals as to the clinical appearance of the disease and periodical ophthalmic tests with mallein are the methods by which the most accurate results of the immunization tests can be obtained. Serum tests in these cases are of little value, as they invariably demonstrate immune bodies or antibodies in the immunized animals, and since even small quantities of mallein injected into a horse are sufficient to produce antibodies which remain for 3 or 4 weeks.

PREVIOUS RESULTS WITH VARIOUS IMMUNIZING AGENTS.

Curative results from mallein were reported by Leclainche, Hueppe, Nocard, Johne, and Wladimiroff, while its immunizing value against glanders was studied by Schindelka, McFadyean, and Semmer, but the results were unsatisfactory. Taking into consideration the literature at our command and drawing conclusions from the results obtained, it appears that mallein possesses very little immunizing value and no great benefit can be expected from its use as a curative agent.

Other investigators attempted to immunize horses and other animals against glanders with the use of killed glanders bacilli and the literature contains some favorable results from this method of immunization. The preparations which were employed for this purpose were in most instances suspensions of glanders bacilli killed by heat. Of the various products which have been prepared and are at the present time used to a limited extent for the immunization of glanders, "farase," so termed by Levy, Blumenthal, and Marxer, gives apparently the best results. It is prepared by killing glanders bacilli with 80 per cent glycerin or 10 per cent urea. The bacilli are then dried and the substance is used in that condition for the immunization. It does not contain living bacteria. Favorable results were obtained with farase by Bautz and Machodin, and by Dediulin. The results of Dediulin are probably the most remarkable, since he reports that on an estate where previous to immunization 276 glandered animals had been destroyed, he injected 303 animals and after one year and

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four months not a single case of glanders developed, although in the meanwhile 14 cases of glanders developed among 300 nonimmunized animals.

Bautz and Machodin subjected farase to various tests to establish its immunizing value. Their results on guinea pigs, cats, and horses were very satisfactory. Guinea pigs which were given two injections of farase resisted six weeks later an intraperitoneal infection with 1/2500 and 1/5000 mg. of glanders bacilli. Of six horses which received two immunizing injections of farase, two were given 1/2500 mg. of glanders bacilli subcutaneously, two received 1/500 mg. of glanders bacilli per os, and two were exposed with the other animals 45 days after the second injection. For each of the groups one check was used. Post-mortem examination of the check animals four to five weeks after the infection showed typical glanders, while the two immunized animals which received subcutaneous injections of glanders bacilli failed to show any lesions of the disease. No record was obtained of the four remaining immunized animals, as they were turned over to another laboratory for study of the duration of immunity in these horses.

One of the recent works on the immunization of glanders was published by Zurkan, who studied the formation of specific antibodies in the blood of horses under the action of glanders antigens. He concludes that of various antigens such as farase, killed glanders bacilli, mallein, and malleo-aggressin, the first and the last (farase and malleo-aggressin) proved most active in the production of immune bodies. The degree of immunity in the animals was established by Zurkan from the comparative results of the serological reactions he obtained with the complement-fixation, agglutination, precipitation, and opsonic tests. Since there were no practical tests made on these animals, his statement that malleo-aggressin may be used for the immunization of horses against glanders can not be accepted as conclusive.

At the meeting of the American Veterinary Medical Association in Indianapolis, MacKellar presented his conclusions on the protective effect of glanders vaccine. The proportion of infections in the stables where these outbreaks occurred, as indicated by the agglutination test, is astonishing. As there is no mention made in the article of the time the agglutination tests were applied subsequent to the mallein test, it suggests that the large proportion of reactors to the agglutination test were the result of the mallein injection and not due to the presence of the infection. If this be true, then the effect of the vaccine remains indefinite and the control of the disease must be accredited to the other precautions which were observed. At best it will require several years before the value of any method of immunization can be satisfactorily established.

EXPERIMENTS WITH DRIED GLANDERS BACILLI.

The New York City board of health has been conducting immunizing experiments with a vaccine prepared in their laboratory, consisting of a suspension of dried glanders bacilli. Each cubic centimeter of the suspension contains 2 mg. of dried bacilli. Through the courtesy of Dr. William H. Park, director of the laboratory, a sufficient quantity of such vaccine was obtained for conducting a series of investigations relative to the possibility of conferring immunity to animals injected with this vaccine.

GUINEA-PIG EXPERIMENTS.

The experiments were made on guinea pigs and on horses. Twenty guinea pigs, about 600 grams in weight, were divided into 4 groups, 4 pigs of each group receiving three immunizing injections of a definite amount of vaccine at intervals of one week. The size of the doses and other details are presented in Table 1. After the conclusion of these vaccinations one pig from each group was subjected to infection with suspensions of glanders bacilli. These injections with infectious material were administered at various intervals. In all instances the same strain of glanders bacilli was used for the infections. The fifth pig in each group was not vaccinated, but served as a check, receiving only a corresponding quantity of glanders bacilli.

The results of these guinea-pig tests showed that there was not a sufficient increased resistance among the vaccinated guinea pigs to warrant any hopes of successful immunization by this method. It is to be regretted, however, that in the infection of these pigs probably too large a quantity of glanders bacilli was used. On the other hand, it would appear that if there had been any appreciable immunity present in the vaccinated guinea pigs they would have manifested it by a greater resistance against the infection.

TABLE 1.—*Immunization tests on guinea pigs with glanders vaccine.*

Guinea pigs.		Vaccination.				Subcutaneous injection of glanders bacilli.		Date of death.	Bacteriological result.
Group.	No.	First injection.	Amounts injected subcutaneously at 7-day intervals.			Date.	Amount.[1]		
			C. c.	*C. c.*	*C. c.*				
1	1	May 19, 1913	0.1	0.3	0.5	June 10, 1913	Dilution in 10 c. c. bouillon	Died June 28, 1913	B. mallei recovered.
	2	do	.1	.3	.5	June 17, 1913	do	Died July 14, 1913	Do.
	3	do	.1	.3	.5	June 24, 1913	Dilution in 20 c. c. bouillon	Died July 28, 1913	Do.
	4	do	.1	.3	5	July 1, 1913	do	Killed July 20, 1913[2]	Do.
	5	Check				June 10, 1913	Dilution in 10 c. c. bouillon	Died July 8, 1913	Do.
2	1	May 19, 1913	.3	.5	.8	do	do	Died July 12, 1913	Do.
	2	do	.3	.5	.8	June 17, 1913	do	Killed July 16, 1913[2]	Do.
	3	do	.3	.5	.8	June 24, 1913	Dilution in 20 c. c. bouillon	Killed Aug. 20, 1913[2]	Do.
	4	do	.3	.5	.8	July 1, 1913	do	Died Aug. 13, 1913	Do.
	5	Check				June 17, 1913	Dilution in 10 c. c. bouillon	Killed June 28, 1913[2]	Do.
3	1	May 19, 1913	.6	.9	1.2	June 10, 1913	do	Died July 16, 1913	Do.
	2	do	.6	.9	1.2	June 17, 1913	do	Died July 24, 1913	Do.
	3	do	.6	.9	1.2	June 24, 1913	Dilution in 20 c. c. bouillon	Killed July 16, 1913[2]	Do.
	4	do	.6	.9	1.2	July 1, 1913	do	Killed July 26, 1913[2]	Do.
	5	Check				June 24, 1913	do	Killed July 5, 1913[2]	Do.
4	1	May 19, 1913	1	1.5	2	June 10, 1913	Dilution in 10 c. c. bouillon	Died July 5, 1913	Do.
	2	do	1	1.5	2	June 17, 1913	do	Died Aug. 2, 1913	Do.
	3	do	1	1.5	2	June 24, 1913	Dilution in 20 c. c. bouillon	Killed Aug. 20, 1913[2]	Do.
	4	do	1	1.5	2	July 1, 1913	do	Died July 8, 1913	Do.
	5	Check				do	do	Died Aug. 3, 1913	Do.

[1] The amount of injection was 0.5 c. c. of suspension in 10 c. c. or 20 c. c. bouillon containing 1 loophole of surface growth from agar culture.
[2] These guinea pigs were killed on account of extensive suppurating abscesses.

EXPERIMENTS ON HORSES.

In the experiments conducted on horses, 17 animals were used, which were purchased on the open market. Most of the animals were aged, but otherwise in fair condition. All the horses were subjected to the agglutination, complement-fixation, and the ophthalmic mallein tests, prior to the vaccination. All of them proved free from glanders on all the tests. Since the amount of vaccine to be injected for immunizing purposes has not been established, it was deemed advisable to employ varying quantities for the injections, and in order to determine the resistance of the animals against infection during and after the vaccination they were subjected to exposure at different times during the investigation.

The smallest amount of the suspension used for the vaccination was the quantity recommended by the New York City Board of Health, viz, 1, 3, and 5 c. c. per injection, while the largest amount any of the horses received was 4, 8, and 12 c. c., respectively. Two of the vaccinated horses received an infection on the nasal mucosa with glanders bacilli, taken up on the end of a platinum loop, one week after the last vaccination. Both of these horses promptly developed glanders and one of them, No. 102, died of an acute form of the disease 21 days after the infection. Thus, there appeared to be no resistance, or at least no increased resistance, against artificial infection.

To establish the resistance of the vaccinated animals against contact infection a corral was built where all the animals, including two artificially infected glanders cases, were kept. They were fed in common feed boxes and were watered from a common trough. Only one hayrack was used for all animals. Simultaneously with this exposure a stable with three stalls was likewise used for exposing the horses. The construction of the stalls in this stable was such that the animal in the center could reach to the feed boxes of either of the horses in the side stalls. The horse placed in the center was a good discharging case of clinical glanders, whereas the horses placed in the side stalls were either two immunized animals or two controls, all of which were given one week's exposure with this infected horse. This was accomplished by changing the horses in the two side stalls every week, and bringing in two others from the corral, so as to make the exposure as uniform as possible in all animals, including the checks. The conditions of exposure were apparently severe, yet they did not exceed the exposure which occurs in the stables of large cities where the sanitary conditions are very poor and where poor light and ventilation afford a splendid opportunity for the propagation of the disease. In fact, the exposure in the corral was rather slight, since the sunlight no doubt had a destructive influence on the infection.

All animals were subjected periodically to clinical examinations and only four of the vaccinated horses developed signs of the disease up to the conclusion of this experiment, although some of them were exposed since May 16. Horse No. 99, which received 4 immunizing injections and was exposed to a discharging case of glanders in the stable, died 15 days after the exposure from acute broncho-pneumonia malleosa.

In order to determine whether any of the vaccinated horses were infected with the latent form of the disease, all were subjected July 23 to the ophthalmic test. This gave surprising results. Two of the vaccinated animals gave a marked reaction (P + + +). A similar reaction was also obtained in the affected horses used for exposure, while of the two check animals which were not vaccinated but had been exposed to a similar extent as the vaccinated animals, only one responded to the test; the other check animal failed to give any reaction. One month later all horses in the experiment were again subjected to the ophthalmic test. The results were the same as on the previous test, but it was noted that the intensity of the reaction was not as pronounced as in the first test. The inflammation and amount of purulent discharge were somewhat less than in the previous test. This observation coincides with that of Meyer, who states that after several eye tests in positive cases of glanders the degree of the reaction becomes less distinct.

The detailed account of the results of the immunizing tests in horses is given in Table 2.

TABLE 2.—*Immunization tests on horses with glanders vaccine.*

Horse No.	Amount and date of vaccination.							Date of exposure.	Ophthalmic tests.			Post-mortem.	Remarks.
	1 c.c.	2 c.c.	3 c.c.	4 c.c.	5 c.c.	8 c.c.	12 c.c.		July 23, 1913.	Aug. 23, 1913.	Jan. 14, 1914.		
	1913.	1913.	1913.	1913.	1913.	1913.	1913.	1913.					
99	Mar. 28		Apr. 4		Apr. 11	Apr. 25		May 16				Acute glanders	Died May 31, 1913.
107	do		do									Impaction	Died Apr. 21, 1913.
86	do		do		Apr. 11	Apr. 25		May 16	−	−	P++	Acute glanders	Killed Jan. 16, 1914.
102	do		do		do			Apr. 18[1]				do	Died May 9, 1913.
111	do		do		do			do	P+++	P++	P+++	Active lesions glanders.	First clinical symptoms of glanders Apr. 23, 1913. Killed Jan. 16, 1914.
110	do		do		do	Apr. 25		May 16	−	−		No glanders	Killed Aug. 20, 1913.
105	do		do		do	do		do	P+++	P++		Glanders nodules in lungs.	Killed Aug. 20, 1913. No clinical signs.
117		May 2		May 9		May 16		do	−	−		Acute glanders	Died Oct. 17, 1913. Clinical symptoms.
118		do		do		do		May 21	P+++	P++		Glanders nodules in lungs.	Killed Aug. 20, 1913. No clinical signs.
119		do		do		do		May 28	−	−		Acute glanders	Died Oct. 25, 1913. Clinical symptoms.
120		do		do		do		May 21	−	−		No glanders	Killed Aug. 20, 1913.
123				May 20		May 27	June 24	June 20	−	−	P+++	Pulmonary glanders	Killed Jan. 16, 1914.
124				do		do	do	do	−	−		No glanders	Killed Aug. 20, 1913.
94[2]								May 16	P+	P+		Glanders nodules in lungs.	Killed Aug. 20, 1913. No clinical signs.
121[2]								May 20	−	−	P+++	Pulmonary glanders	Killed Jan. 16, 1914.
82[3]									P+++	P+++		Glanders nodules in lungs, liver, and lymph glands.	First clinical symptoms of glanders Mar. 26, 1913, and continued to progress. Died Oct. 15, 1913.
122[4]									P+++	P++			First clinical symptoms of glanders May 26, 1913, and continued to progress. Died June 16, 1913, of flatulent colic.

[1] Infected.
[2] Not vaccinated.
[3] Infected Mar. 21, 1913, and used to expose other horses.
[4] Infected May 22, 1913, and used to expose other horses.

AGGLUTINATION AND COMPLEMENT-FIXATION TESTS.

In order to study the effect of the immunizing injections on the serum tests, the blood of the horses in this experiment was subjected to the agglutination and complement-fixation tests from the time of the first injection until the conclusion of the work. It was found that the agglutination value of the serum of the vaccinated horses as a rule increased from the third day after the first vaccination and continued to rise for a time. A decrease was again noted from two to four weeks after the last vaccination and appeared practically normal after six weeks to two months. A complement fixation with the sera of the vaccinated horses was obtained from the seventh to the ninth day after the first vaccination and they continued to give positive fixations from two to three months after the last vaccination.

These negative serological results which followed the positive reactions due to the injected vaccine, appeared only in the animals which gave no reaction to the ophthalmic test, while the blood of those vaccinated horses which gave a positive reaction to the eye test continued to give a positive fixation until they had been destroyed and proved to be affected with the disease. The same condition was observed in the animals which had been artificially infected with glanders.

The serological results from these investigations appear to have a great significance with reference to the immunity produced by the injection of dead glanders bacilli. The fact that the demonstration of the presence of immune bodies in the vaccinated horses ceased entirely in two or three months from the last vaccination would indicate that after the lapse of such a time the animals have very little or no immunity against the disease. This is further substantiated also by the agglutination value of the sera returning to the normal level. As a matter of fact, previous investigations carried out by Dr. Buck, of this laboratory, showed that one or two subcutaneous injections of mallein will give a complement fixation which may last from one to two months. The agglutination value of the serum of such animals is also markedly influenced by subcutaneous malleinization. The serum reaction of horses following the subcutaneous injections of mallein is given in detail in Table 4. From this it seems that a mallein injection has almost the same action on the production of immune bodies in a horse as killed glanders bacilli. Table 3 indicates the results obtained with the agglutination and complement-fixation tests in the animals used in this investigation.

On August 20 two vaccinated horses and one check animal which gave positive results to the eye test were destroyed, and in all three animals marked pulmonary glanders was observed. Horse No. 105 showed the presence of glanders nodules in the lungs in very great numbers, some of which were of the size of a walnut. In the two other cases, while the nodules were very numerous and from their

appearance appeared to be active, they were of smaller sizes, ranging from a pinhead to the size of a pea. Horses Nos. 110, 120, and 124 were killed on the same day, although they had failed to show any indication of glanders by the eye test, which was also substantiated by the complement-fixation test with the blood of these animals. Post-mortem examination showed no signs of glanders in these animals.

The final results are quite striking relative to the deficiency of immunization against glanders by killed bacteria.

Of the remaining horses which were kept under observation, as indicated in Table 2, Nos. 117 and 119 died on October 17 and 25, respectively, of acute glanders after developing the clinical form of the disease. No. 86 also showed indications of the disease in the early part of October. The final test on the remaining horses, namely, Nos. 86, 111, 121, and 123, was undertaken in the early part of January, 1914, when they were subjected to the ophthalmic and subcutaneous mallein tests and also to the complement-fixation and agglutination tests. All horses reacted to these different tests with the exception of No. 86, which reacted to the fixation, agglutination, and ophthalmic tests but failed to react to the subcutaneous mallein test. Two days following the tests all the animals were destroyed and careful post-mortem examinations were made. The results showed glanders lesions in all animals, including No. 86, in varying degrees. The lungs in all cases contained numerous glanders nodules most of which were in an active stage, and in horse No. 86 the apex of the left lung showed a typical glanderous pneumonia with the characteristic gelatinous infiltration and numerous nodules of various sizes throughout the remainder of the pulmonary tissue.

It is interesting to note that all these vaccinated horses returned to the normal serum reaction of a negative case on or before the twelfth week after the vaccination, as may be seen from Table 4. The exposure in the corral was continued the same as during the entire course of the experiments and the weekly changes of stable exposure were also carried out. The appearance of the disease in these remaining animals seems to offer a more substantial basis for drawing conclusions as to the unsatisfactory results of these vaccination tests.

From our experience with outbreaks of glanders in stables, it appears that these experimental horses did not develop clinical manifestations of the disease in greater proportion than is the case with the average exposed horse. It is true that the exposure of the horses in the experiments was continuous although not unusually or unreasonably severe. Whether the horses which were included in this final test possessed a certain degree of immunity as a result of the vaccination, during the period including the time between the first exposure and the last negative eye test, it is impossible to say; but

TABLE 3.—*Serum reactions in horses vaccinated against glanders.*

Horse No.	Vaccination period.	Agglutination. During vaccination period. At first vaccination.	Agglutination. During vaccination period. Second week.	Agglutination. During vaccination period. Fourth week.	Agglutination. After vaccination. Second week.	Agglutination. After vaccination. Fourth week.	Agglutination. After vaccination. Sixth week.	Agglutination. After vaccination. Eighth week.	Agglutination. After vaccination. Twelfth week.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. During vaccination period. At first vaccination.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. During vaccination period. Second week.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. During vaccination period. Fourth week.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. After vaccination. Third week.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. After vaccination. Sixth week.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. After vaccination. Eighth week.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. After vaccination. Tenth week.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. After vaccination. Twelfth week.	Complement fixation with 0.1 c.c. and 0.2c.c. of serum. After vaccination. Oct. 14, 1913.	Remarks.
99	Mar. 28 to Apr. 25	1:400—	1:2,000+	1:2,000+	1:2,000+	.....	.....	.....	.....	—	+	+	+	.....	.....	.....	.....	.....	Died May 31 from acute glanders and influenza.
86	.....do.....	1:400—	1:2,000+	1:2,000+	1:1,500	1:1,000	1:800	1:400—	1:400—	—	+	+	—	—	-	.....	.....	+	Killed Jan. 16, 1914. Post-mortem showed acute glanders.
110	.....do.....	1:400—	1:1,500	1:2,000+	1:2,000+	1:1,000	1:400—	1:400—	1:400—	—	+	+	+	+	—	—	.....	.....	No glanders. Killed Aug. 20, 1913.
105	.....do.....	1:400	1:2,000	1:2,000+	1:2,000+	1:1,500	1:800	1:2,000	1:2,000+	.....	+	+	+	+	+	—	+	.....	Post-mortem showed pulmonary glanders.
117	May 2 to May 16	1:400—	1:2,000	1:2,000+	1:2,000+	1:1,000	1:400	1:400—	1:400—	—	+	+	+	—	-	—	.....	+	Died Oct. 17, 1913. Post-mortem showed acute glanders.
118	.....do.....	1:400	1:2,000	1:2,000+	1:1,500	1:1,500	1:800	1:1,500	1:2,000	—	+	+	+	—	+	—	+	.....	Post-mortem showed pulmonary glanders.
119	.....do.....	1:400—	1:1,500	1:2,000+	1:1,500	1:1,000	1:400	1:400—	1:400	—	+	+	+	+	-	-	.....	+	Died Oct. 25, 1913. Post-mortem showed acute glanders.
120	.....do.....	1:400	1:2,000	1:2,000+	1:2,000+	1:1,500	1:800	1:400	1:400	.....	+	+	+	-	.....	.....	.....	.....	Post-mortem showed no signs of glanders.
123	May 20 to June 24	1:400	1:2,000	1:2,000+	1:2,000	1:1,500	1:1,000	.....	1:400	.....	+	+	+	-	.....	.....	.....	+	Killed Jan. 16, 1914. Post-mortem showed pulmonary glanders.
124	.....do.....	1:400	1:1,500	1:2,000	1:2,000	1:1,500	1:800	.....	1:400	.....	+	+	+	.....	.....	.....	.....	.....	Post-mortem showed no signs of glanders.
94	Unvaccinated check infected by exposure.	[1] 1:400—	[2] 1:400	[2] 1:800	[2] 1:1,500	[2] 1:2,000	[2] 1:1,500	[2] 1:800	[2] 1:1,000	[1]	[2] +	[2] +	[2] +	[2] +	[2] +	[2] +	[2] +	.....	Post-mortem showed pulmonary glanders.
121	Unvaccinated check remained healthy.	[1] 1:400	[2] 1:400	1:400	[2] 1:400	[2] 1:400	[2] 1:400	[2] 1:400	[2] 1:400	[1]	[2] +	[2] +	[2] +	[2] +	[2] +	[2] +	[2] +	+	Killed Jan. 16, 1914. Post-mortem showed pulmonary glanders.

[1] Before exposure.

[2] After exposure.

TABLE 4.—*Serum reactions in normal horses following the subcutaneous injection of 1 cubic centimeter of mallein.*

Horse No.	Test.	Jan. 31, 3 days.	Feb. 3, 6 days.	Feb. 6, 9 days.	Feb. 9, 12 days.	Feb. 13, 16 days.	Feb. 17, 20 days.	Feb. 20, 23 days.	Feb. 23, 26 days.	Feb. 27, 30 days.	Mar. 3, 34 days.	Mar. 6, 37 days.	Mar. 10, 41 days.	Mar. 13, 44 days.	Mar. 17, 48 days.	Mar. 21, 52 days.	Mar. 24, 55 days.
96	Complement fixation	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2
		— —	— —	−S1+	−S1+	S1+½+	S1+S1+	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
	Agglutination	1:500−	1:800	1:800	1:800	1:800	1:500	1:600	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−
98	Complement fixation	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2
		— —	— —	−S1+	¾+ +	+ +	+ +	¾+¾+	¾+¾+	S1+½+	S1+½+	S1+½+	S1+½+	S1+S1+	S1+S1+	— —	— —
	Agglutination	1:800	1:2,000	1:1,500	1:1,500	1:2,000	1:2,000	1:1,500	1:2,000	1:1,500	1:1,000	1:1,000	1:1,000	1:800	1:800	1:500−	1:500−
102	Complement fixation	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2
		— —	— —	— —	−S1+	S1+½+	S1+½+	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
	Agglutination	1:600	1:1,000	1:1,000	1:1,000	1:1,000	1:800	1:1,000	1:800	1:800	1:600	1:800	1:600	1:600	1:500−	1:500−	1:500−
103	Complement fixation	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2
		— —	— —	— —	— —	+ +	+ +	¾+¾+	¾+¾+	½+¾+	½+¾+	½+½+	S1+¼+	S1+½+	S1+S1+	−S1+	— —
	Agglutination	1:500−	1:800	1:1,000	1:1,000	1:800	1:800	1:600	1:500	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−
107	Complement fixation	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2
		— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
	Agglutination	1:800	1:1000	1:1,500	1:1,500	1:2,000	1:1,500	1:1,500	1:1,000	1:1,000	1:1,000	1:1,000	1:1,000	1:800	1:800	1:800	1:500−

SERUM REACTIONS FOLLOWING A SECOND INJECTION OF 1 CUBIC CENTIMETER OF MALLEIN MAR. 28, 1913.

Horse No.	Test.	Mar. 31, 3 days.	Apr. 3, 6 days.	Apr. 7, 10 days.	Apr. 10, 13 days.	Apr. 14, 17 days.	Apr. 17, 20 days.	Apr. 21, 24 days.	Apr. 24, 27 days.	Apr. 28, 31 days.	May 1, 34 days.	May 5, 38 days.	May 8, 41 days.	May 12, 45 days.	May 15, 48 days.	May 19, 52 days.	May 22, 55 days.	June 2, 67 days.
96	Complement fixation	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2	0.1 0.2
		— —	S1+S1+	S1+S1+	½+½+	S1+S1+	S1+S1+	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —	— —
	Agglutination	1:500−	1:500−	1:600	1:800	1:500−	1:500−	1:600	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−	1:500−
98	Complement fixation	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2
		— —	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	½+¾+	½+¾+	½+¾+	½+¾+	½+½+	— —
	Agglutination	1:800	1:1,500	1:2,000	1:2,000	1:1,500	1:1,500	1:1,500	1:1,000	1:800	1:600	1:600	1:600	1:600	1:600	1:600	1:500−	1:500−
103	Complement fixation	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2	.1 .2
		— —	¾+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	+ +	¾+¾+	½+¾+	½+¾+	½+½+	½+½+	— —
	Agglutination	1:600	1:1,500	1:2,000	1:2,000	1:2,000	1:2,000	1:2,000	1:1,000	1:1,000	1:800	1:800	1:800	1:800	1:800	1:600	1:500−	1:500−

Quantities of serum employed in complement-fixation tests, 0.1 c. c. and 0.2 c. c. Fractions represent the approximate degree of fixation as compared with complete fixation S1+ indicates slight fixation.

Six dilutions of sera employed in agglutination test; 1:500, 1:600, 1:800, 1:1,000, 1:1,500, and 1:2,000.

from the sanitarian's standpoint this would be of theoretical importance only, since even if such should be the case an immunity of from two to four months could not be considered sufficient for practical vaccination purposes. Furthermore, it should be remembered that some of these horses developed a latent form of the disease in less than three months from the last vaccination during the period in which the blood still contained the so-called immune bodies.

CONCLUSIONS.

The results obtained by these investigations appear to be sufficient to demonstrate the unsatisfactory results of this method of immunization. Of the 13 immunized animals, 9 contracted the disease from natural exposure, which is a large proportion when it is considered that all animals were aged and kept most of the time during the exposure out of doors. Of the 4 remaining immunized horses, 1 died of impaction after the second vaccination, while the other 3 animals were killed August 20, 1913, in order to ascertain by post mortem examination the possibility of glanders existing in these animals which had given positive serum reaction, but which had returned to normal. In artificial infections of the vaccinated animals they showed no resistance whatsoever, as both vaccinated horses promptly developed an acute form of the disease from touching the Schneiderian membrane with a platinum loop which had been touched to a growth of glanders bacilli. For the present, therefore, it seems advisable to abstain from immunizing horses by this method, as a practice of this kind may do more harm than good. Owners having horses which are supposedly immunized would naturally become careless, thinking their animals were resistant to the disease, and thus even a better opportunity would be offered for the propagation of the disease than if the horses were not vaccinated. Furthermore, the fact that the blood of vaccinated animals can not be utilized for serum tests for two or three months after the injections is also a great disadvantage in the eradication of the disease.

As a result of this preliminary work it appears that the control and eradication of glanders must still be dependent upon the concentration of our efforts in eliminating infected horses and the adoption of proper precautions against the introduction of infected animals into stables free from the disease. The results achieved in Germany, Austria, and Canada by these methods have proved very encouraging, and no doubt if executed in the same spirit in this country a marked reduction in the cases of glanders would result.

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